CHARACTERISTICS OF THE RETINAL ELECTRIC RESPONSE OF THE OCELLI OF LIMULUS

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The median ocelli of *Limulus polyphemus*, located one on each side of a spine on the dorsal and anterior surface of the carapace, are well suited for investigations into the electro-physiological properties of photoreceptors. These small clusters of sense cells, about 50–80 in number, can be dissected free of the cornea and surrounding connective tissue quite readily. The free ocellus, about 0.5 mm. in diameter, can then be drawn into a tapering capillary tube. Such a preparation, providing it is not subjected to unreasonable abuse, will give reproducible electrical responses upon illumination for periods up to six hours.

The data presented below were obtained from ocelli treated in the manner just described. A capillary containing the ocellus was sealed into a small hole in the partition which separated two compartments of a plastic electrode chamber. The light-receiving end of the ocellus was in contact with sea water of the forward compartment and the terminal portion of the nerve was in contact with sea water in the rear compartment. Each compartment contained a silver-silver chloride electrode in contact with the sea water.

The light source used in this experiment was a 500 watt tungsten filament projection bulb. At the focal point the maximal intensity was 27,000 foot candles and this was considered unit intensity. To cool the light beam it was passed through 8 centimeters of water and a heat-absorbing filter. The intensity of the light beam was reduced by Wratten neutral tint filters. The duration of the light flash was controlled by a photographic shutter. A portion of the light beam was diverted to a photocell, the output of which was recorded as the stimulus signal. The nominal exposures of the shutter were calibrated by photographing a cathode ray beam deflected by the output of the photocell.

The potentials appearing across the eye on illumination were fed into high gain, condenser-coupled amplifiers and recorded on an ink writer. The recording paper speed was 25 centimeters per second; the paper speed was calibrated with a 60 cps. signal. The ink writer responded faithfully to calibrating signals up to 60 cycles per second. The deflections of the writing pens were calibrated with square wave voltage pulses and a 60 cycle per second signal after each exposure. The time constant of the amplifiers was 0.5 second. It is felt that the distortion of the wave form of the retinal action potentials caused by the 0.5 second time constant does not seriously affect the results obtained nor the interpretation of the results.

After dark-adaptation was complete, a flash of light of the lowest intensity to be used was admitted and the response recorded. For relatively low intensities and/or short durations (less than 0.1 second) of illumination, 10 minutes between flashes

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were found to be sufficient for recovery. For higher intensities and/or for longer durations (more than 0.1 second) of illumination, it was necessary to allow 15 to 20 minutes between flashes.

Results

The wave form of the response

The time course of the retinal action potential obtained upon brief illumination of an excised median ocellus is indicated in Figure 1. The smooth contour of the response is similar to the second response curve obtained from the lateral eye of

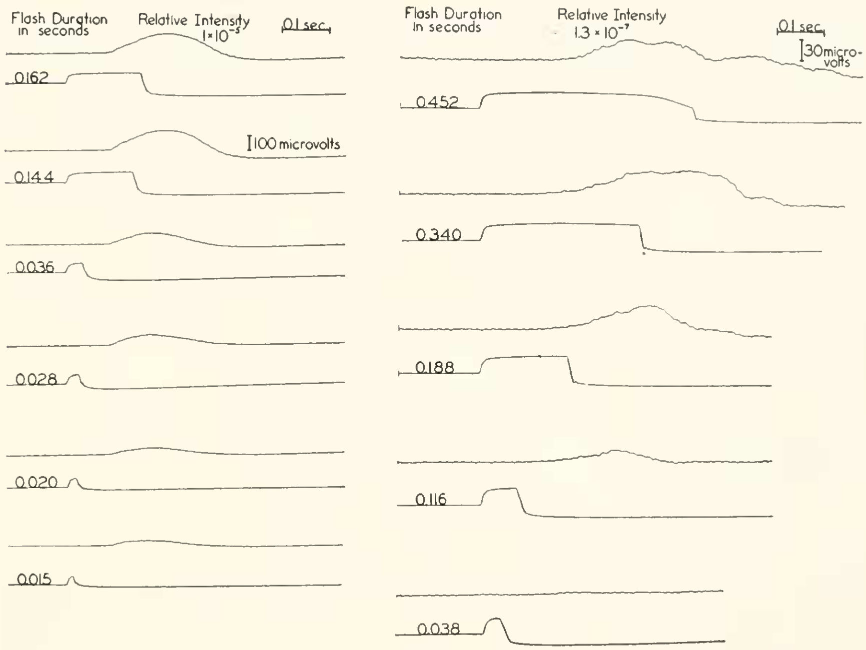

FIGURE 1. Right: Photographs of retinal action potentials elicited from an isolated ocellus illuminated with low intensity for varying durations. The duration in seconds is given on the lower tracing of each pair of records, which is the record of the output of the photocell. The ocellus was maximally dark adapted before each flash.

Left: Photographs of retinal action potentials from an ocellus illuminated with a moderate intensity for varying durations. Records marked as above. Refer to text for further detail.

Limulus (Graham, 1932; Wulff, 1950). Light flashes of very low intensity (0.027 f.c. or less) often elicit responses of irregular contour (Fig. 1, right). As the flash duration is increased, the response spreads out in time and the irregularity increases. If the intensity of illumination is increased, the irregularity of the response disappears.

The effect of light flashes of constant duration and variable intensity

A light stimulus of the lowest useful intensity generally elicits a response with crest magnitudes of about 10 microvolts. As the intensity of illumination is increased, the response magnitude increases also, the relation being such that the

Table I

Latent periods and crest times obtained during a typical experiment with an excised median ocellus of Limulus. Relative intensities are at the top of each column. All times are in milliseconds. The means and their standard deviation are given underneath particular columns

Flash Duration msec.	1×10^{-6}		1×10^{-5}		1×10^{-4}		1×10^{-3}		1×10^{-2}		1×10^{-1}		1×10^{0}	
	Latent Period	Crest Time	Latent Period	Crest Time	Latent Period	Crest Time	Latent Period	Crest Time	Latent Period	Crest Time	Latent Period	Crest Time	Latent Period	Crest Time
17	128	96	80	80	72	92	52	84	40	72	28	36	23	32
21	120	60	85	92	73	83	56	76	40	64	29	35	23	32
32	118	94	89	112	68	84	56	84	—	—	28	32	23	32
42	104	100	88	124	72	84	56	88	40	68	—	—	—	—
101	108	125	91	120	71	77	56	92	40	76	—	—	—	—
256	100	200	84	129	74	82	—	—	—	—	—	—	—	—
Mean	113		86		72	84	55	85		70	28	34		
Standard Deviation	±9.8		±3.6		±1.9	±4.4	±1.6	±4.8		±6.3	±0.6	±1.6		

magnitude increases linearly with the logarithm of the intensity. The largest response magnitude recorded from an ocellus exposed to a flash of unit intensity (27,000 f.c. at the surface of the ocellus) was 5 millivolts.

The latent period of the electric response generally decreases as the intensity increases. The behavior of both magnitude and latent period of the response of the ocellus are in agreement with similar characteristics of the lateral compound eye of Limulus (Wulff, 1950).

The effect of light flashes of constant intensity and variable duration

When the intensity of the light stimulus is maintained constant and the duration is increased, a series of responses such as is illustrated in Figure 1, left, may be

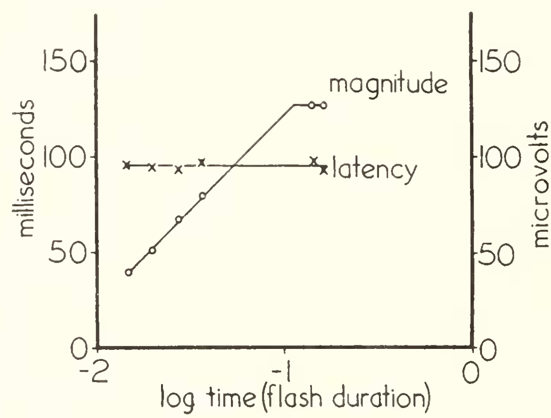

Figure 2. Magnitude of the crests and latencies of the responses of Figure 1, left, plotted as a function of the common logarithm of the flash duration. Refer to text for further detail.

obtained. The latent period of the responses is constant for all flash durations at the same intensity level (Fig. 1 and Table I). It is only when the intensity level is changed that the latent period changes.

The magnitude of the responses illustrated in Figure 1, left, gradually increases to a maximal value which is reached when the flash duration is about 0.1 second (see Fig. 2). Light flashes of duration longer than 0.1 second do not produce any further increase in magnitude. The influence of flash duration on response magnitude has been determined for the grasshopper eye by Hartline (1928).

Histological examination of ocelli

Ocelli used in these experiments were prepared for histological study by fixing in Bouin's solution, imbedding in paraffin, sectioning and staining with thionine ($C_{12}H_9N_3S$). This stain has a marked affinity for the spherical rose-colored inclusions richly distributed throughout the cytoplasm of the sense cells. In suitable preparations cut in cross section, the numbers of sense cells were counted. The results indicate that ocelli of the size used in these experiments contain between 50 and 80 sense cells.

Discussion

Although the electrical response of the ocellus of Limulus does not differ significantly from the electrical responses of other photoreceptors, certain of the observations lead to a critical evaluation of some of the characteristics of the retinal action potential.

The magnitude of the retinal action potential

The increase in the crest magnitude of the retinal action potential of the dark-adapted photoreceptor, produced by increasing the intensity of illumination or by increasing the duration of exposure at a given intensity, has been frequently reported (Kohlrausch, 1931; Hartline, 1928; Granit, 1947; Wulff, 1950). The growth of response magnitude seems to be characteristic of photoreceptors, yet no adequate explanation has been advanced to account for the tremendous range of responses of which photoreceptors are capable. The observations reported here suggest that the increase in magnitude of the electrical response of the median ocellus of Limulus may be primarily the result of graded responses on the part of the sense cells, with the added possibility that sense cell recruitment may occur at low intensities of illumination.

That the sense cells of the median ocellus of Limulus can produce responses of graded magnitude is indicated by the following observations. The maximal response recorded from an isolated ocellus was of the order of 5 millivolts. Minimal response magnitudes that could be measured with reliability were of the order of 0.01 millivolt. Should 0.01 mv. represent the maximal (all or none) response of a single sense cell then, assuming the best possible conditions for summation, the number of sense cells required to produce a response of 5 mv. would be 500. Actual counts of sense cells in ocelli used range between 50 and 80, a number entirely inadequate to account for the observed results unless each sense cell is capable of producing a response whose magnitude varies with the intensity, the duration of illumination or both.

The recruitment of sense cells by light flashes of long duration incident on an excised median ocellus of Limulus is illustrated in Figure 1, right. The extreme temporal dispersion of discrete electrical waves indicated in Figure 1 is observable only at low levels of illumination. Increasing the intensity of illumination generally produces a smooth response regardless of the exposure (Fig. 1, left). However, the crests of the smooth responses shift in time as the light flashes increase in duration so that the crest time, measured from onset of response to the crest, increases (Table I). The shift in crest time is apparent only at the lower intensities of illumination (Table I) and it is assumed that recruitment of sense cells occurs at those intensities where the crest time increases with increasing flash duration.

The retinal action potential and the optic nerve discharge

It has been suggested (Hartline, 1935; Wulff, 1943; Granit, 1947) that the retinal action potential generates local currents which initiate impulses in the optic nerve. In the light of such an hypothesis a large retinal action potential would set up more intense local currents than a smaller potential and hence the resultant nerve impulse discharge might begin sooner in time and be more intense, *i.e.,* the frequency of the impulses might be greater. Hartline (1934) has measured the characteristics of the nerve impulses discharged into a single optic nerve fiber in response to light flashes of different intensities and durations. The results of Hartline's observations that pertain to this discussion are: 1) The frequency of the first three impulses of the train of impulses increases with increasing flash duration, reaching a value that is roughly constant at flash durations of 0.1 second and higher; 2) The latency of the optic nerve discharge decreases as the flash duration increases, reaching values roughly constant for flash durations of 0.1 second and higher. Assuming for the moment that the characteristics of the ocellus of Limulus are applicable to the lateral eye, then the results of Hartline's experiment are in accord with the hypothesis that the retinal action potential initiates activity in the optic nerve because: 1) the direction of the changes in latency and frequency of the optic nerve discharge produced by light flashes of increasing duration is in harmony with the increase in magnitude of the retinal action potential; and 2) the salient characteristics of both the retinal action potential and the optic nerve discharge are constant for light flashes of durations of 0.1 second and higher. Very recently it was demonstrated that the response characteristics of the lateral eye of Limulus are in accord with the characteristics of the ocellus.

The constant latent period

The results reported above indicate that (within the limits of exposures available) the latent period of the retinal action potential is an inverse function of the intensity of illumination and is quite independent of the duration of illumination. Since the magnitude of the response increases to a maximal value at any given intensity for flash duration of 0.1 second or greater and the latent periods of the responses are quite constant (Table I), the possibility is suggested that the onset of the response and the magnitude of response may be controlled by separate events in the sense cells. The validity of this suggestion must be tested by further investigation.

Another interpretation of these results may be based on the existence of a sensitization period, first described by Hecht (1918, 1919). According to Hecht, the sensitization period is that period during which the photoreceptor must be illuminated in order to produce a response. The duration of such a sensitization period in the Limulus ocellus might well be shorter than the shortest flash duration available, and it is conceivable that this sensitization period may determine the latency, *i.e.,* at any given intensity, a response of fixed latency either will occur or else there will be no response at all. Data obtained from the ocellus of Limulus at low levels of illumination (Fig. 1) indicate that the response magnitude for short exposures tends to approach the abscissa (zero microvolts) very gradually; there is no suggestion of a sudden drop that might be expected if a sensitization period actually exists.

Summary

1. The electrical responses to illumination were obtained from isolated median ocelli of *Limulus polyphemus* mounted in a capillary tube filled with sea water. These preparations, maintained in a state of dark adaptation, were periodically illuminated at different intensities for different durations.

2. For the most part, the reported data are in accord with similar data obtained from the lateral compound eye of Limulus and the compound eyes of grasshoppers. It was observed that the latent period of the responses elicited by light flashes of different durations at any one intensity is constant; the only factor seemingly affecting the latent period is the intensity.

3. The increase in electrical response magnitude produced either by increasing the intensity and/or the duration of illumination, which is typical of many photoreceptors, is accounted for on the basis of graded responses on the part of sense cells. The possibility exists that recruitment of sense cells is a contributing factor at low intensities of illumination.

4. Comparison of data obtained from the median ocellus of Limulus with data obtained from single optic nerve fibers of the lateral eye of Limulus indicates a parallelism which is in accord with the hypothesis that the retinal action potential sets up local action currents which initiate impulses in the optic nerve.

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